Table 1. Effect of betamethasone disodium phosphate on the incorporation of [³H]proline into protocollagen of carrageenin granuloma in rats*

Treatment	No. of rats	Radioactivity of [3H]hydroxyproline			
		Before incubation (dis./min)	After incubation (dis./min)	Radioactivity of [³ H]hydroxyproline formed (dis./min)	Inhibition (%)
Control	7	108 :- 30	661 ± 53	553 + 29	
Betamethasor	ne 8	17 🚣 8	271 ± 22	254 ± 26	54.0

^{*} Results are shown as means \pm S.E. Treatment of rats and incubation conditions are described in the text.

synthesis of the collagen by inhibiting protocollagen synthesis without affecting the hydroxylation of protocollagen. The findings described above, however, do not rule out the possibility that the steroid inhibits [3H]proline transport through the cell membrane and affects the proline pool size of the fibroblast, since all of the results described above were obtained from the experiments analyzing the incorporation of [3H]proline into protocollagen or collagen hydroxyproline.

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Effect of oxytocin on Mg2+-dependent ATPase

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In addition to various ATPases in erythrocyte membrane there is also present Mg²⁺-dependent ATPase (adenosine triphosphate hydrolase, Mg²⁺—EC 3.6.1.4) the function of which is not clear yet. Certain relation between this enzyme, the membrane contractile protein and the contractile protein from smooth muscle is supposed. It was viz proved that the Mg²⁺-dependent ATPase in erythrocyte membrane is activated by adrenaline or noradrenaline, i.e. by the hormones which influence the contraction of smooth muscle. In the present paper an attempt was made to determine whether oxytocin which influences the contraction of some smooth muscles will activate Mg²⁺-dependent ATPase in erythrocyte membrane, too.

Materials and Methods

0.1 ml of packed erythrocytes from freshly drawn human blood were hemolyzed by 2 ml of aqueous ATP solution either with or without oxytocin, the buffer was added to the hemolyzate. The final volume of incubation solution was 3 ml and the final concentration of individual substances was as follows; 1.07 mM ATP, 0.16 M Tris buffer, 2.65 mM MgCl₂ and 5×10^{-4} M ouabain; pH of the incubation mixture was 7.4. After 1 hr incubation at 37° the amount of inorganic phosfate (P) was determined according to Fiske and Subbarow.³ The activity of Mg²⁺-dependent ATPase was expressed by the amount of P in nmoles splitted from added ATP by 0.1 ml of packed cells after 1 hr of incubation at 37° .

When determining the erythrocyte hemolysis 0·1 ml of packed erythrocytes were incubated for 1 hr at 37° with 3 ml of 200 mOsm NaCl either with or without oxytocin. The amount of hemoglobin (Hb) in supernatant after the centrifugation of samples was determined by the method of Crosby and Furth.⁴ The synthetic oxytocin of firm Spofa was used.

Results

After the incubation of erythrocytes with different amount of oxytocin the activity of Mg²⁺-dependent ATPase rised with increasing concentration of the hormone and was highest with the highest concentration used, i.e. 5 IU on 0·1 ml of erythrocytes. The dependence between the enzyme activity and oxytocin concentration was not linear (Fig. 1).

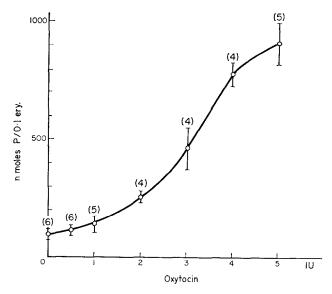


Fig. 1. The influence of oxytocin on the activity of Mg²⁺-dependent ATPase in crythrocytes. Mean values with S.E. are given from the number of experiments noted in parentheses. The activity of Mg²⁺-dependent ATPase was expressed by the amount of P in nmoles splitted from added ATP by 0·1 ml of packed cells after 1 hr of incubation at 37°.

The addition of oxytocin up to the amount of 3 IU to 0·1 ml of erythrocytes had no hemolyzing effect, evident hemolysis of erythrocytes was only in the presence of 4 and particularly of 5 IU on 0·1 ml of erythrocytes. After the addition of 4 IU of oxytocin 0·042 mg Hb resp, 0·084 mg Hb were released and after the addition of 5 IU of oxytocin 0·156, resp, 0·462 mg Hb were released from 0·1 ml of erythrocytes.

Discussion

Oxytocin in the amount higher than 1 IU per 0·1 ml of packed erythrocytes increased the activity of Mg²+-dependent ATPase and in the amount higher than 3 IU it hemolyzed erythrocytes, presumably by interfering with the structure of membrane. The above mentioned effect of oxytocin on hemolysis of erythrocytes is not in contradiction to its effect on mitochondria—in vitro it causes their swelling.⁵

One can see that the hormone oxytocin which is known for its physiological influence mainly on uterus has at high concentration also a significant influence on Mg²⁺-dependent ATPase in erythrocyte membrane. As high doses of oxytocin are required for inducing the mentioned effects on erythrocyte membrane the specificity of its effect may be disputable. On the other hand one can understand that a hormone which under physiological condition influences in the human body particularly uterus and breast can influence erythrocytes only at the high concentration. From the results given one cannot decide whether oxytocin influence the membrane Mg²⁺-dependent ATPase directly or indirectly by means of another component of the membrane.

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